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Nat Rev Neurosci. Author manuscript; available in PMC 2011 April 01.

Published in final edited form as:
Nat Rev Neurosci. 2010 October ; 11(10): 682–696. doi:10.1038/nrn2911.

Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders

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Abstract

There is a long-standing paradox that N-methyl-D-aspartate receptors (NMDARs) can both promote neuronal health and kill neurons. Recent studies show that NMDAR-induced responses depend on the receptor location: stimulation of synaptic NMDARs, acting primarily through nuclear Ca^{2+} signaling, leads to the build-up of a neuroprotective 'shield', whereas stimulation of extrasynaptic NMDARs promotes cell death. These differences result from the activation of distinct genomic programmes and opposing actions on intracellular signalling pathways. Perturbations in the balance between synaptic and extrasynaptic NMDAR activity contribute to neuronal dysfunction in acute ischaemia and Huntington's disease and could be a common theme in the aetiology of neurodegenerative diseases. Neuroprotective therapies should aim to both enhance the effect of synaptic activity and disrupt extrasynaptic NMDAR-dependent death signalling.

Introduction

NMDARs (N-methyl-D-aspartate (NMDA) receptors) are cation channels that are gated by the neurotransmitter glutamate. They are essential mediators of many forms of synaptic plasticity and also mediate aspects of development and synaptic transmission[1, 2]. In addition to their synaptic localization, NMDARs are found at extrasynaptic and perisynaptic sites, which can be defined immunohistochemically (e.g. distal from the post-synaptic density, PSD) and electrophysiologically (e.g. out of reach of normal synaptic glutamate release, see Box 1). In immature hippocampal neurons, extrasynaptic NMDARs can represent up to three quarters of all NMDARs[3]. Although the proportion of synaptically located NMDARs increases with development, a significant population of NMDARs remain extrasynaptic in adulthood[3-6]. The exact location of extrasynaptic NMDARs had not been thoroughly addressed until recently[6]. Only around one quarter of extrasynaptic NMDARs in adult hippocampal slices are classified as being perisynaptic (that is, within 100 nm of the PSD), the rest being localized on dendrites or on non-perisynaptic parts of the spine. Of the dendritically localized extrasynaptic NMDARs, around one third are adjacent to glia-like processes, and around half are adjacent to axons. The physiological function of extrasynaptic NMDARs is not fully understood, but their activation by glutamate spillover may contribute to long-term depression [7-9]. This Review will focus on the emerging role of extrasynaptic NMDARs in pathological scenarios, in contrast with the demonstrated survival-promoting effects of synaptic NMDAR activity.

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We first discuss the background to the field of excitotoxicity, the dichotomous nature of NMDAR signalling, and the evidence that receptor location plays a role in this dichotomy. We then outline the molecular mechanisms that underlie synaptic NMDAR-dependent neuroprotection and the various pathways that are coupled preferentially to extrasynaptic NMDARs. After discussing the potential basis for differential signalling by synaptic versus extrasynaptic NMDARs we review recent studies that address the relevance of synaptic versus extrasynaptic NMDAR signalling in acute and chronic neurological disorders, focusing on stroke and Huntington's disease. Finally we examine prospects for therapies designed to selectively target extrasynaptic NMDAR signalling while sparing synaptic signalling.

The 'NMDAR paradox'

The neurotoxicity of sustained glutamate exposure to retinal neurons was first reported over 50 years ago[10], at around the same time as the excitatory properties of glutamate were being characterized[11]. Later, Olney showed that glutamate toxicity was not restricted to retinal neurons, and coined the term "excitotoxicity" [12]. Choi subsequently demonstrated that Ca^{2+} entry was a key mediator of glutamate excitotoxicity, and that the NMDAR was the primary source of toxic Ca^{2+} influx [13-15]. Shortly after, it was proposed that Ca^{2+} entry through NMDARs was particularly effective at killing neurons compared to entry through other channels [16]. At the same time, evidence was accumulating that implicated glutamate toxicity and NMDAR activity in acute neurological trauma such as stroke, as well as circumstantial evidence pointing to a role for glutamate toxicity and NMDAR activity in chronic neurodegenerative diseases, including Huntington's disease [17-19]. Since then, the work of many laboratories have advanced our understanding of how glutamate dyshomeostasis, ionic imbalance and abnormal NMDAR activity can contribute to such disorders [19-24].

The destructive effects of NMDAR activity are in striking contrast to the observation that the survival of several types of neuron is dependent on synaptic NMDAR activity and function[25-27]. Elimination of NMDAR activity *in vivo* causes widespread apoptosis and enhances trauma-induced injury in developing neurons[28-32]. In the adult CNS, NMDAR blockade exacerbates neuron loss when applied after traumatic brain injury (TBI) or during ongoing neurodegeneration[33], and impairs the survival of newborn neurons in the adult dentate gyrus[34]. Thus, NMDARs are important for cell survival but can also be harmful and kill neurons. For many years it was thought that the degree of Ca^{2+} entry though NMDARs was solely responsible for these differences in cellular outcome: moderate levels of NMDAR activity were considered to be beneficial for neurons, whereas excessive activation of NMDARs, with subsequent 'Ca^{2+} overload' of the neurons, was deleterious. According to this model, neuronal responses to NMDAR activity followed a bell-shaped curve, where both too much and too little was potentially harmful[35-37].

This view may be subject to revision in light of recent work that has revealed an alternative explanation for the 'NMDAR paradox', namely that it is the location of the NMDAR that also influences whether it is coupled to pro-death or pro-survival signals. According to this new model, synaptic NMDARs are neuroprotective, whereas extrasynaptic NMDARs preferentially initiate cell death pathways. Thus, the fate of neurons is not determined solely by the degree of overall NMDAR activity but by the extent to which synaptic and extrasynaptic NMDARs are activated. This may be best described with an 'χ-shaped' graph in which an ascending curve that represents increased neuroprotection due to increased synaptic NMDAR activity is superimposed on a descending curve that illustrates the progressive decrease in neuroprotection due to increasing extrasynaptic NMDAR activity (Figure 1). According to this concept, it is not the 'Ca^{2+}-overload' *per se* that is the sole determinant of toxicity but rather the Ca^{2+} flow though NMDARs located outside the

synapse that is particularly harmful to neurons. Synaptic NMDAR activity is by nature phasic and extrasynaptic activation is achieved by chronic agonism, but when equivalent overall Ca^{2+} concentrations are triggered by synaptic and extrasynaptic NMDARs, they cause strikingly different downstream events[38]. Ca^{2+} influx evoked by intense synaptic NMDAR activation is not only well tolerated by hippocampal neurons (i.e. it causes no perturbations to mitochondrial membrane potential), it is also the trigger for genomic processes that render neurons more resistant to apoptotic and oxidative insults[38] (see below). In sharp contrast, comparable Ca^{2+} transients induced by activation of extrasynaptic NMDARs — either on their own or in the presence of synaptic NMDAR activation — trigger mitochondrial dysfunction and cell death[38].

The distinct synaptic versus extrasynaptic NMDAR signalling pathways that lead to survival and death, respectively, were initially characterized in hippocampal neurons, and subsequently were confirmed to exist in cortical neurons[39]. Recent studies have shown that a selective activation of extrasynaptic NMDARs in hippocampal neurons triggers the same amount of cell death as activation of all NMDARs (synaptic and extrasynaptic) despite the fact that extrasynaptic NMDARs elicit a smaller initial Ca^{2+} elevation[40], highlighting the importance of extrasynaptic NMDARs in excitotoxicity. Moreover, several studies have demonstrated that certain effects of extrasynaptic NMDAR signalling dominate over effects of synaptic signalling[38], [41]-[43]. However, under chronic conditions of NMDAR activation, the balance between synaptic and extrasynaptic NMDAR activity is important [44] and can influence the point at which escalating levels of bath-applied NMDA or glutamate become neurotoxic. For example, bath application of low concentrations of NMDA can induce firing activity and so preferentially activate synaptic pathways and activity-dependent neuroprotection, whereas higher levels of NMDA suppress firing, resulting in extrasynaptic pathways dominating [45].

Synaptic NMDAR-dependent neuroprotection

According to the theory of neuronal health[46], neurons can exist in a spectrum of states ranging from fully functioning and resilient at one extreme to dysfunctional and vulnerable to insults at the other extreme. The position of the neuron within this spectrum is influenced by a multitude of signals and stimuli, but is known to be shifted towards health and robustness by synaptic NMDAR activity. An episode of synaptic NMDAR activity promotes neuroprotection that lasts beyond the duration of the episode and continues after most signalling pathways are no longer active[47]. This long-lasting form of ‘acquired neuroprotection’ predominantly results from changes in gene expression that have effects at multiple levels within the cell (Figure 2) such as enhancement of mitochondrial health, boosting of antioxidant defences and suppression of caspase activation, all of which in turn serve to preserve neuronal function and improve viability in the face of insults.

Induction of survival genes

Ca^{2+} signalling is a major mediator of the dialogue between the synapse and cell nucleus. It has been known for over 20 years that seizure activity triggers transcriptional responses in neurons [48], as do physiological patterns of synaptic NMDAR activity, such as those that promote synaptic potentiation [49]. Ca^{2+} entry through synaptic NMDARs is augmented by release from internal stores, after which Ca^{2+} is transduced to the cell soma where it invades the nucleus [50]. Nuclear Ca^{2+} is one of the most potent activators of neuronal gene expression. Transcriptome analyses have revealed that in hippocampal neurons nearly 200 genes are controlled by nuclear Ca^{2+} signalling[42], [51].

One important target of nuclear Ca^{2+} is the nuclear Ca^{2+}/calmodulin-dependent protein (CaM) kinase IV and the transcription factor cyclic-AMP response element binding protein

(CREB, Figure 3a–pathways A-D)[52-58]. CREB is the prototypical signal-regulated transcription factor, whose role in neuronal survival, as well as in several other processes (including synaptic plasticity, addiction, neurogenesis, and learning and memory), is well documented[59-62,63]. In hippocampal neurons, CREB-dependent gene expression is causally linked to the long-lasting phase of activity-dependent neuroprotection against apoptotic and excitotoxic insults[47, 64]. This form of acquired neuroprotection is dependent on nuclear Ca^{2+} signalling[47], which is consistent with the known role for nuclear Ca^{2+} in CREB activation[50, 57, 65]. In recent years, the genomic programmes that underlie acquired neuroprotection have been uncovered[42, 51]. Among the pool of nuclear Ca^{2+}-regulated genes, a core set of ten genes, termed *Activity-regulated inhibitors of death* (*AID*) genes have been identified, which have been shown to provide neurons with a broad-spectrum neuroprotective 'shield', both in cell culture and in animal models of neurodegeneration[51]. Some of the *AID* genes, which include the genes encoding activating transcription factor 3 (*Atf3*), B-cell translocation gene 2 (*Btg2*), B-cell lymphoma 6 (*Bcl6*), growth arrest and DNA damage induced gene 45 beta (*GADD45beta*), growth arrest and DNA damage induced gene 45 gamma (*GADD45gamma*), Inhibin beta-A (*Inhba*), Interferon activated gene 202B (*Ifi202B*), neuronal PAS domain protein 4 (*Npas4*), nerve growth factor IB (also known as nuclear receptor subfamily 4, group A, member 1) (*Nr4a1*), and serine protease inhibitor B2 (*Serpinb2*), seem to be potential CREB target genes and may provide neuroprotection through a common process that renders mitochondria more resistant to cellular stress and toxic insults (Figure 4a)[42, 51, 66, 67]. Another target of synaptic NMDAR and nuclear Ca^{2+}-CREB signalling is the gene that encodes the neurotrophin brain-derived neurotrophic factor (*Bdnf*)[38, 51, 68, 69]. BDNF has neuroprotective properties[70] and can rescue neurons from NMDAR blockade-induced neuronal death[71].

Although CREB is a key mediator of nuclear Ca^{2+}-dependent neuroprotection, other transcription factors implicated in mediating NMDAR-dependent survival may also be partly controlled by nuclear Ca^{2+}, such as nuclear factor of activated T-cells (NFAT[72]). By contrast, NMDAR-dependent activation of other neuroprotective factors such as nuclear factor I, subtype A (NFI-A) might occur independently of nuclear Ca^{2+} elevation (its activation being dependent on the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and nitric oxide (NO) synthesis[73]).

Suppression of death genes

A second mechanism through which synaptic NMDAR activity restricts the apoptotic potential of a neuron involves the transcriptional suppression of core components of the intrinsic apoptosis cascade (Figure 4a)[66, 67]. Synaptic NMDAR activity suppresses the expression of the pro-apoptotic Bcl-2 homology domain3 (BH3)-only member gene *Puma in vitro* and *in vivo*[66, 67]. *Puma* expression is both sufficient to induce cell death and necessary for apoptotic death in response to NMDAR blockade[67]. As with activation of CREB, suppression of *Puma* is specific to synaptic NMDAR signalling, as bath activation of all NMDARs (synaptic and extrasynaptic) fails to influence *Puma* expression. Suppression of *Puma* inhibits the apoptotic cascade upstream of cytochrome c release, but auxiliary protective mechanisms also exist downstream, as the genes encoding the core apoptosome components apoptotic protease activating factor 1 (Apaf-1) and pro-caspase-9 are also subject to transcriptional suppression by synaptic NMDAR activity[66, 67]. The net effect of all these transcriptional changes is that the activation of caspase-9 and downstream executioner caspases is very limited, apoptosis is inhibited, and the neurons remain viable and electrically active [67].

Synaptic NMDAR activity also suppresses the expression and/or activity of important pro-death transcription factors (Figure 3b, Figure 4a). The forkhead box O (FOXO) class of transcription factors can promote neuronal death following excitotoxic injury, trophic factor

withdrawal and oxidative stress[74-77]. Known pro-death FOXO target genes include the BH3-only genes *Noxa*, Bcl2-like protein 11 (*Bcl2l11*, also known as *Bim*) and *Puma*, Fas ligand (*Fasl*) and thioredoxin-interacting protein (*Txnip*). In conditions of trophic deprivation, in which FOXOs are constitutively located in the nucleus, synaptic NMDAR activity promotes sustained activation of the Akt pathway, leading to the phosphorylation and nuclear export of FOXO and the subsequent inactivation of FOXO target genes, which includes *Foxo1* (*Foxo1* itself was recently identified as a FOXO target gene[43, 78], Figure 3b–pathway A). Furthermore, synaptic NMDAR activity induces a long-lasting inhibition of FOXO nuclear import[77] (Figure 3b–pathway D). This long-lasting blockade was shown to require nuclear CaM kinase IV activation and *de novo* gene expression, although the exact mechanism by which this leads to blockade of FOXO nuclear import remains unclear. Thus, synaptic NMDAR activity both promotes the nuclear export of FOXOs and the transcriptional suppression of *Foxo1* and places a long-lasting blockade on FOXO nuclear translocation (Figure 3b). *p53* is another pro-apoptotic transcription factor that is subject to negative transcriptional regulation by synaptic NMDAR activity and that may contribute to the suppression of *Apaf-1*[66], although p53-independent routes to *Apaf-1* expression also exist[67].

Protection against oxidative stress

In addition to apoptosis-suppressing effects, synaptic NMDAR activity also enhances antioxidant defences[43] (Figure 2, Figure 4b), which contributes to neuroprotection against oxidative insults. NMDAR blockade *in vivo* triggers neuronal death that is associated with protein carbonylation (a marker of oxidative stress), which is suggestive of a role for physiological NMDAR activity in supporting antioxidant defences. Synaptic NMDAR activity also controls neuronal vulnerability to oxidative stress-induced death *in vitro*[43]. It triggers a number of changes to the thioredoxin-peroxiredoxin system that contribute to this neuroprotective effect. Specifically, synaptic activity enhances thioredoxin activity and facilitates the reduction of hyperoxidized peroxiredoxins, which are an important class of antioxidant enzymes. These effects are mediated by a coordinated programme of gene expression changes, some of which have been characterized[43]. These changes include the transcriptional suppression of the FOXO target gene *Txnip*, which is dynamically regulated by NMDAR activity *in vitro* and *in vivo*. Also, enhanced reduction of hyperoxidized peroxiredoxins is associated with transcriptional activation of two genes, *Srxn1* (sulfiredoxin) and *Sesn2* (sestrin 2). The products of these genes can mediate this reaction[79-81], with sulfiredoxin induction likely to be more important[82, 83]. Trans-synaptic stimulation of synaptic NMDARs is crucial for boosting antioxidant defences, as chronic bath activation of all (synaptic and extrasynaptic) NMDARs failed to trigger the aforementioned transcriptional changes or protect against oxidative insults.

The gene expression changes (Figure 4b) do not account for all the antioxidant effects of synaptic NMDAR activity, raising the possibility that other antioxidant systems, such as the glutathione system, may also be subject to control by synaptic NMDAR activity.

Pro-death signalling by extrasynaptic NMDARs

NMDAR-dependent neuron death is mediated by a variety of pathways, the relative contribution of which may depend on the cell type, the developmental stage or intensity of the excitotoxic insult[20, 84]. Recent studies have found that certain pro-death pathways or events are preferentially activated by extrasynaptic NMDARs compared to synaptic ones (Figure 5). As discussed below, in some cases this involves direct antagonism of synaptic NMDAR-activated survival pathways, whereas in others it involves the triggering of events/pathways that are simply not activated by synaptic NMDARs.

CREB shut-off

Bath activation of NMDARs has long been known to be a poor activator of CRE-dependent gene expression in hippocampal neurons, compared to Ca^{2+} entry though voltage-gated Ca^{2+} channels (although activation of the serum response element (SRE) was equally good)[85]. This could be explained by the fact that bath activation of NMDARs induces CREB-activating phosphorylation of CREB at serine-133, followed shortly afterwards by a strong CREB-dephosphorylation signal [86], [87]. By contrast, activation of voltage-gated Ca^{2+} channels induces sustained CREB phosphorylation [86]. The fact that NMDARs seemed to be poor inducers of CRE-dependent gene expression when they were bath-activated was at apparent odds with the fact that NMDARs were known to induce activation of CRE-dependent gene expression following electrical activity [49], [88]. These historical observations can be explained by the observation that synaptic NMDAR activity promotes sustained CREB phosphorylation, whereas extrasynaptic NMDARs are coupled to a dominant CREB dephosphorylating signalling pathway [38], [89] (Figure 3a). When all (synaptic and extrasynaptic) NMDARs are activated by bath-applied glutamate or NMDA, the extrasynaptic pathway dominates, resulting in weak CREB activation [38], [85], [86]. The only exception is when low levels of bath-applied NMDA induce firing activity, in which case synaptic signalling dominates and CREB activation is relatively strong [45].

Recently, insight into the mechanistic basis of this differential signalling was uncovered and found to be centred on Jacob, a binding partner of the neuronal Ca^{2+} binding protein caldendrin[90]. Jacob, when localized to the nucleus, causes CREB dephosphorylation and also promotes a loss of synaptic contacts. Jacob contains a nuclear localization signal and its nuclear import requires importin-α binding. This binding and nuclear import is prevented by caldendrin, which binds Jacob in a Ca^{2+}-dependent manner. Synaptic NMDAR activity was shown to promote caldendrin-dependent retention of Jacob outside of the nucleus, whereas extrasynaptic NMDAR activity promoted the nuclear accumulation of Jacob, with subsequent effects on neuronal survival [90] (Figure 3a–pathway F). As the coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated and absent in very young neurons [89], it will be of interest to know whether there is a developmental switch that activates the Jacob pathway.

ERK1/2 inactivation

The ERK1/2 pathway, which can support activation of CREB and inactivate the pro-death protein Bcl-2-associated death promoter (BAD) has been implicated in NMDAR-dependent neuroprotection,[26]. Synaptic NMDAR activity promotes sustained ERK activity[47], [91], whereas activation of all NMDARs by bath application of NMDA results in ERK activation that is followed by inactivation[91]-[93]. The basis for this bi-directional control of ERKs turned out to be due to the opposing actions of synaptic versus extrasynaptic NMDARs[41]. Synaptic NMDARs couple to activation, whereas extrasynaptic NMDARs promote ERK dephosphorylation and subsequent inactivation in both hippocampal[41] and cortical neurons[39] (Figure 3a–pathway E). Consistent with this, extrasynaptic NMDARs strongly inactivate Ras (upstream of ERK) [93]. Thus, synaptic and extrasynaptic NMDARs are mutually antagonistic with regard to Ras-ERK signalling. However, ERK1/2 inactivation by extrasynaptic NMDAR is stimulus-specific: although extrasynaptic NMDARs exert an ERK1/2 shut-off signal that is dominant over that triggered by activation of synaptic NMDARs, it does not antagonize ERK1/2 activity induced by BDNF[94].

FOXO activation

Synaptic NMDAR activity inactivates pro-death FOXOs at multiple levels (see above and Figure 3b), whereas extrasynaptic NMDAR activity does the opposite. Chronic activation of all (synaptic and extrasynaptic NMDARs) does not result in FOXO export from the nucleus,

because activation of Akt is not sustained[43] (Sustained activation of the Akt pathway leads to the phosphorylation and nuclear export of FOXO). More recently, it was shown that extrasynaptic NMDAR-evoked signals not only fail to induce FOXO nuclear export, but instead promote the nuclear import of FOXOs that are normally sequestered in the cytoplasm[77] (Figure 3b–pathway C). This activation of FOXOs subsequently contributes to NMDAR-dependent neuronal death. Thus, the activation status of FOXOs is highly sensitive to the balance between synaptic and extrasynaptic NMDAR activity (Figure 3b).

Calpain activation and STEP cleavage

Activation of extrasynaptic NMDARs strongly activate calpains, whereas synaptic NMDAR activity does not[95]. In agreement with this, the classical calpain substrate fodrin and Na+/Ca^{2+} exchanger 3 (NCX3)[95] are cleaved following extrasynaptic, but not synaptic, NMDAR activity. NCX3 is known to be cleaved by calpains in animal models of stroke, leading to delayed Ca^{2+} deregulation and neuronal death[96]. Another substrate for extrasynaptic NMDAR-activated calpains is striatal enriched tyrosine phosphatase (STEP), which is cleaved into a form that cannot interact with its normal substrates. One such substrate is p38 mitogen-activated protein (MAP) kinase, which is negatively regulated by STEP, is enriched at extrasynaptic membrane fractions, and is known to contribute to death induced by chronic NMDA or glutamate exposure in cerebellar granule cells and cortical neurons[95, 97-99]. Inhibition of calpain-mediated STEP cleavage is sufficient to inhibit NMDAR-dependent p38 activation and can protect neurons from glutamate toxicity and oxygen-glucose deprivation[95]. Thus calpain — coupled to STEP cleavage and subsequent p38 activation — represents another pro-death cascade that selectively activated by extrasynaptic NMDARs (Figure 5). One important caveat to the role of p38 in excitotoxicity is that its influence on survival and death is heavily context-dependent. For example, it can promote neuroprotection through activation of myocyte enhancer factor 2 (MEF2) [100] and indeed can be activated by neuroprotective, synaptic NMDAR activity [99, 101]. Thus, its contribution to neuronal death in excitotoxicity may rely on interactions with other events that are triggered by the excitotoxic insult.

The observation that p38 is disinhibited by extrasynaptic NMDAR activity through STEP cleavage also raises the question as to why extrasynaptic NMDARs fail to activate ERK1/2, as STEP is also a powerful negative regulator of ERKs[95, 102]. It is possible that other, dominant ERK-inactivating signals may be triggered in parallel (such as those discussed above), overriding any effects of STEP cleavage.

Gene expression changes specifically induced by extrasynaptic NMDAR activity

As a result of these or other differences in signalling, extrasynaptic NMDAR activity induces a very different programme of gene expression compared to synaptic NMDARs[42]. As new gene expression contributes to at least some forms of excitotoxic neuronal death, these changes are likely to be functionally important. Although the identity of such genes remains to be elucidated, they may include FOXO target genes or other factors targeted by extrasynaptic signals. For example, *Clca1*, the gene that encodes Ca^{2+} -activated chloride channel regulator 1, is selectively activated by extrasynaptic NMDAR activity in cell culture and in a stroke model, and its overexpression is sufficient to kill neurons[42, 103], although the mechanism by which its expression is induced is unclear.

New developments and ongoing questions

Control of nuclear architecture by synaptic and extrasynaptic NMDARs

In addition to differentially controlling specific transcription-regulating events, synaptic and extrasynaptic NMDARs may also more globally affect signal processing towards and within

the cell nucleus by regulating nuclear architecture. A structural analysis of hippocampal nuclei using a novel algorithm for three-dimensional reconstruction uncovered that - in contrast to what is commonly thought and what is depicted in virtually all textbooks - many cell nuclei are not round or near-spherical organelles but have a very complex, highly infolded shape [104]. The structure of the nucleus is dynamic and signal-regulated, with synaptic and extrasynaptic NMDARs having opposing effects: stimulation of synaptic NMDARs increases the number of infolded nuclei, whereas the infolded shape is rapidly lost following Ca^{2+} flux through extrasynaptic NMDARs. Thus, neurons translate NMDAR signals into changes in nuclear geometry, providing a means for an activity-dependent modulation of nucleo-cytoplasmic exchange of molecules and ions [104]. The changes in nuclear shape and consequent rearrangements of the nuclear architecture and spatial re-organization of chromosome territories may affect chromatin structures and accessibility and could have profound effects on gene expression and genome stability. In addition, infolded and near-spherical nuclei have similar volumes but infolded nuclei have a larger surface and larger number of nuclear pore complexes [104]. Given that actively transcribed DNA segments may be located near nuclear pore complexes [105], [106], a possible increase in the number of nuclear pore complexes in infolded nuclei following synaptic NMDAR activation may enhance the capacity of the nucleus for transcription-coupled anchoring of promoter regions to nuclear pore complexes. Synaptic NMDAR-induced structural plasticity of the nucleus may therefore represent an adaptation to a metabolically more active state with an increased demand for gene expression.

The basis for differential synaptic versus extrasynaptic NMDAR signalling: outstanding questions

The differences in signalling between synaptic and extrasynaptic NMDARs could theoretically be due to one of three factors, or a combination thereof. Firstly, they could be due to differences in the make-up of the NMDAR signalling complexes at extrasynaptic compared to synaptic locations. The components that make up the NMDAR signalling complex in the post-synaptic density are the subject of intense study[107], but the molecules that associate with extrasynaptic NMDARs have been poorly characterized. Some proteins that are found in synapses and that are linked to synaptic NMDARs, such as the membrane-associated guanylate kinases (MAGUKs) postsynaptic density protein (PSD)-95 and PSD-93, discs, large homolog 1 (Drosophila) (DLG1, also known as SAP97, and discs, large homolog 3 (Drosophila) (DLG3, also known as SAP102), are also linked to extrasynaptic NMDARs. In 3-week old hippocampal cultures, around 20% of extrasynaptic NMDARs were found to be associated with PSD-95 and a further 20% with SAP102[6]. Association of extrasynaptic GluN1 NMDAR subunits with MAGUKs was also observed by double-labelling immuno-gold electron microscopy in adult hippocampal slices [6]. This is of particular interest since MAGUKs have been implicated in coupling the NMDAR to downstream death pathways such as excessive nitric oxide production [108], [109]. Other studies suggest the possibility of a specific association of extrasynaptic NMDARs with certain proteins. For example, it has been postulated that, as caldendrin is localised to the post-synaptic density, only trans-synaptic activation of synaptic NMDARs trigger local Ca^{2+} transients that are high enough to promote caldendrin-mediated Jacob retention[90]. Alternatively, the preferential localization of p38 (but not ERK1/2) at extrasynaptic membranes may explain its preferential activation by extrasynaptic NMDAR-dependent STEP cleavage[95]. It is likely that other, yet to be identified proteins associate differentially with synaptic/extrasynaptic NMDARs.

Secondly, receptor subunit composition could play a role in the differences in signalling between synaptic and extrasynaptic NMDARs. The cytoplasmic C-termini of the GluN2 subunits have undergone considerable sequence divergence through evolution, which

potentially allows for differential association of signalling molecules. During development GluN2B is the predominant subunit at all synaptic and extrasynaptic locations in forebrain neurons, and GluN2A expression in the rodent CNS begins at the end of the first post-natal week[110, 111]. In the adult forebrain both the GluN2A and GluN2B subunits are prevalent. GluN2A becomes incorporated into synaptic NMDARs [112] and has been reported to become enriched at synapses compared to extrasynaptic locations, with GluN2B more abundant at extrasynaptic locations[113, 114]. No studies, however, have demonstrated a clear-cut spatial demarcation. Indeed, GluN2A subunits have been identified at extrasynaptic sites in cultured neurons[115] and the subunit compositions of synaptic and extrasynaptic NMDARs in 3-week-old acute hippocampal slices have been found to be very similar[116]. Moreover, immunofluorescence and immuno-gold studies have also identified both GluN2A and GluN2B at extrasynaptic locations[6].

Nevertheless, subunit differences could have a contributing effect if GluN2A- and GluN2B-containing NMDARs are coupled differently to pro-survival and pro-death pathways. Indeed, GluN2B or GluN2A-containing NMDARs have been proposed to promote neuronal death and neuronal survival, respectively[117]. However, the specificity of the GluN2A-specific antagonist used in this study[117] has been questioned, particularly when employed *in vivo* or in non-steady state conditions[118-122]. Moreover, it has been shown that GluN2B- and GluN2A-containing NMDARs can both promote survival and death[43, 114, 123]. Whether they do so with differing efficiencies, however, remains unclear as a direct comparison has yet to be made. Indeed, attempting to do so would be challenging given the lack of a sufficiently selective GluN2A-specific antagonist. Also, the fact that GluN2B and GluN2A are expressed at different relative levels in development can cloud the issue, as developmental changes in NMDAR signalling can be misinterpreted as being due to a switch in GluN2 subunit when there are in fact many other potential explanations. Another important issue is that receptors can exist in a tri-heteromeric form that contains both a GluN2A and a GluN2B subunit [124], meaning that 2A- and 2B-dependent signals can theoretically be transduced from the same channel. Whether the GluN2 subunit (and particularly its C-terminus) influences whether an activated NMDAR will promote either survival or death will require more sophisticated approaches in order to be fully resolved.

A final potential factor contributing to differential signalling could be the way in which synaptic and extrasynaptic NMDAR pools are activated. Synaptic NMDARs are generally transiently and intensely activated by the trans-synaptic release of glutamate, while extrasynaptic NMDARs are typically activated chronically, by elevated levels of ambient glutamate. Subtle differences in the spatial properties of intracellular Ca^{2+} transients evoked by these stimuli could differentially affect signalling, even if the overall Ca^{2+} load were similar. If trans-synaptic versus chronic activation of NMDARs is a contributing factor in differential synaptic vs. extrasynaptic NMDAR signalling, this raises the question as to whether synaptic NMDARs can contribute to excitotoxic neuronal death under certain circumstances (e.g. chronic activation). Against this idea is the observation that prior blockade of synaptic NMDARs was not protective against NMDA-induced death [40]. Nevertheless, it is a possibility that under certain conditions where extrasynaptic NMDARs are chronically active, that chronic activation of synaptic NMDARs may contribute to a toxic Ca^{2+} load. Conversely, it is unclear whether extrasynaptic NMDARs can contribute to neuroprotective signalling under certain circumstances. For example, if strong synaptic activity results in the transient trans-synaptic activation of a portion of perisynaptic or extrasynaptic receptors (as has been shown [125]), does this contribute to neuroprotective signalling or antagonize those promoted by synaptic NMDAR activation? It is also not clear what proportion of a cell's extrasynaptic NMDAR population need to be activated in order to promote pro-death signalling.

Another, related question is whether extrasynaptic NMDARs at different locations (dendritic, perisynaptic, cell body) signal differently. Studies have shown NMDARs demonstrating mobility between synaptic and extrasynaptic locations [113], [151], [154] (see Box 1), and it remains to be determined whether these receptors alter their signalling properties upon switching their location. One possibility is that an NMDAR moving (for example) between an extrasynaptic to a synaptic site, encounters a different biochemical environment and so associates with a different complement of downstream signalling molecules. However, the extent to which NMDARs are mobile in vivo remains to be clarified in the light of recent observations that synaptic and extrasynaptic NMDARs from stable pools in acute slices [116].

Addressing the above questions will enhance our knowledge about the molecular basis for differential synaptic vs. extrasynaptic NMDAR signalling, and enhance our understanding of the precise conditions that determine the balance between protective and destructive NMDAR signalling.

Clinical implications

Extrasynaptic NMDARs in ischaemic stroke

Inappropriate levels of NMDAR activity can contribute to neuronal death or dysfunction in a number of paradigms, including acute excitotoxic insults (e.g. stroke, TBI) and certain neurodegenerative diseases, such as HD and AD (reviewed in[23], [24], [126]). Recent work has specifically implicated extrasynaptic NMDAR activation in the aetio-pathophysiology associated with an acute and a chronic form of neuronal dysfunction (ischaemic stroke and HD, respectively).

During an ischaemic episode, extracellular glutamate accumulates due to synaptic release and impairment or reversal of uptake mechanisms[22], [127], and neurons become depolarized, relieving the Mg^{2+} blockade of NMDARs. These events have the combined effect of causing strong activation of NMDARs[128], possibly including those located outside the synapse. The consequences of reversed uptake can be studied in culture through the use of the transportable glutamate transport inhibitor L-trans-pyrrolidine-2,4-dicarboxylate (PDC), which causes reversed glutamate uptake through hetero-exchange[129]. In neuron/astrocyte co-cultures, PDC treatment causes glutamate efflux and excitotoxicity due to an activation of extrasynaptic NMDARs that mainly arises from reversed astrocytic uptake[130]. Interestingly, a significant proportion of extrasynaptic NMDARs *in vivo* are located adjacent to glia[6], raising the possibility that astrocytic glutamate release may lead to activation of these receptors.

A recent study has demonstrated that extrasynaptic NMDAR activity may be selectively enhanced in ischaemia via an intracellular signalling cascade[131]. Ischaemia recruits death-associated protein kinase (DAPK1) directly to the C-terminal tail of GluN2B, leading to phosphorylation of the channel and increased single-channel conductance. The effect is specific to GluN2B-containing NMDARs and results in a selective enhancement of extrasynaptic NMDAR currents[131], which is not observed in DAPK1-deficient neurons. Moreover, peptide-mediated disruption of DAPK1–NMDAR interactions was shown to protect neurons against oxygen-glucose deprivation *in vitro* and ischaemic damage *in vivo*[131]. Taken together, these studies indicate that extrasynaptic NMDAR activity contributes to ischaemic neuron loss.

Synaptic and extrasynaptic NMDARs in Huntington's Disease

Recent studies show that the relationship between extrasynaptic NMDAR signalling and mutant Huntingtin (mtHtt) may be a key factor in HD progression[44], [132] (Figure 6a). One

study focused on the presymptomatic YAC128 transgenic mouse, which expresses a mutant huntingtin (mtHtt) that contains 128 CAG repeats, as compared to a YAC18 control mouse which expresses Htt with a non-pathological CAG repeat length[132]. Although expression of mtHtt had no effect on synaptic NMDAR activity, YAC128 transgenic mice showed both an increase in extrasynaptic NMDAR current and in extrasynaptic NMDAR expression specifically in striatal medium spiny neurons. This enhanced extrasynaptic NMDAR activity was dominated by GluN2B-containing NMDARs and persisted in aged YAC128 mice[132]. Consistent with the previously mentioned coupling of extrasynaptic NMDAR activity to CREB dephosphorylation and inactivation [38], reduced CREB phosphorylation was observed in the striatum of YAC128 mice[132].

In addition to the influence of mtHtt on extrasynaptic NMDAR function, a recent study has demonstrated that synaptic and extrasynaptic NMDAR signalling have distinct and opposing effects on the neurotoxicity of mtHtt itself. Synaptic NMDAR activity reduces mtHtt toxicity by increasing the formation of non-toxic mtHtt inclusions by a process involving the transcriptional up-regulation of the chaperonin TRiC (T complex-1 ring complex) subunit TCP1. TCP1 knock-down or blocking of synaptic activity reduced the formation of inclusions, rendering neurons vulnerable to mtHtt-induced neuronal death. This mtHtt-induced neuronal death is itself dependent on extrasynaptic NMDAR activity[44], which is consistent with the upregulation of extrasynaptic NMDAR currents that have been observed in YAC128 mice[132] and, indeed, with the large body of literature implicating excessive NMDAR-dependent activity in HD pathology[23].

In addition to the enhancement of extrasynaptic NMDAR activity by mtHtt and in mediating mtHtt-induced excitotoxic death, extrasynaptic NMDARs may also serve as up-stream regulators of mtHtt toxicity[44]. Extrasynaptic NMDAR activity was found to support the expression of Rhes, a small GTPase that controls the sumoylation of mtHtt, a modification that promotes mtHtt toxicity and prevents its aggregation[133].

Part of the toxic effects of mtHtt and excessive extrasynaptic NMDAR activity in HD pathology may be attributable to the suppression of pro-survival CREB target gene expression. As stated above, extrasynaptic NMDAR activity is coupled to a CREB-inactivating dephosphorylation signal[38]. Consistent with this, CREB phosphorylation levels are reduced in the striatum of YAC128 mice (where extrasynaptic NMDAR activity is elevated)[132]. Also, mtHtt can suppress CREB-mediated gene expression by sequestering the CREB coactivator CBP — an effect that contributes to mtHtt toxicity[134, 135]. A key neuroprotective CREB target gene whose underexpression is implicated in HD pathogenesis is the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1a (PGC-1α)[136]. In neurons, PGC-1α regulates both mitochondrial density and antioxidant defences, and controls vulnerability to excitotoxic insults by these or other mechanisms[101, 137, 138]. MtHtt interferes with CREB-dependent PGC-1α expression, and this leads to mitochondrial dysfunction and metabolic defects[139, 140]. Indeed, PGC-1α expression is lower in the striatum of human HD patients, but not in the cerebellum or hippocampus, which is consistent with the brain areas associated with HD pathology. As a further exacerbating factor, the striatum seems to be particularly vulnerable to loss of PGC-1α expression[141]. Overexpression of PGC-1α protects both cortical and striatal neurons from mtHtt- and 3-nitropropanoic acid-induced cell death, and lentiviral transduction of PGC-1α into the striatum reduces striatal loss in a mouse model of HD[44, 138-140].

Synaptic activity acts as a positive regulator of PGC-1α expression, both directly — via CREB activation — and indirectly — by inhibiting mtHtt-dependent suppression of PGC-1α (due to the aforementioned TCP1-dependent aggregation of mtHtt)[44, 101]. Moreover, synaptic activity enhances PGC-1α's transactivating capacity through a post-

translational, p38-dependent mechanism[101]. Taken together, the balance between synaptic and extrasynaptic NMDAR activity controls the toxicity of mtHtt and is itself controlled by mtHtt (Figure 6a). This reciprocal, feed-forward control may play a key role in HD progression and indicates that a selective blockade of extrasynaptic NMDARs and sparing of synaptic NMDAR activity offers a potentially attractive therapeutic strategy, as outlined below[44, 132].

Therapeutic targeting of extrasynaptic NMDARs

Despite evidence from animal studies that have implicated NMDARs in ischaemic brain damage, clinical trials of NMDAR antagonists for stroke and TBI have failed due to poor tolerance and efficacy[25, 142]. The important role of NMDARs in the CNS may mean that for many antagonists the maximum tolerated dose may not be therapeutically effective, as many unacceptable side-effects are not off-target effects but are due to blockade of synaptic NMDAR activity. Evidence that NMDAR activity can exert a neuroprotective effect has led to suggestions that it may even promote recovery in the post-reperfusion phase, and prevent delayed neuronal loss in the penumbra[25, 143]. Dichotomous signalling by NMDARs is also evident in juvenile TBI. Here, treatment with NMDAR antagonists reduces primary excitotoxic death but exacerbate secondary apoptosis, resulting in increased overall death[30]. In adult TBI, the NMDAR has also been proposed to rapidly switch between 'destructive' and 'recovery' roles[144, 145]. As such, antagonists may be protective in early stages of the insult progression but not later, highlighting the need for more specific anti-excitotoxic strategies in which the physiological and neuroprotective roles of NMDARs are considered. One promising strategy is the use of antagonists that preferentially block NMDARs in excitotoxic scenarios and of pathologically-activated therapeutics, which are activated only in the pathological state[146]. As an example of the former, memantine is a clinically well-tolerated NMDAR antagonist with pharmacological properties that are suited to the blockade of chronic NMDAR activity in pathological situations without interfering with normal synaptic function[146].

Memantine is an open channel blocker with a fast off-rate. Its uncompetitive nature results in an effective blockade of chronic extrasynaptic NMDAR activity that is triggered by elevated levels of glutamate[126]. Interestingly, due to its fast off-rate and voltage-dependent binding properties memantine, when used at low doses, does not accumulate in the channel and so does not substantially interfere with normal synaptic NMDAR activity [126, 147]. As such, it was proposed that low doses of memantine would not substantially affect the trans-synaptic activation of synaptic NMDARs while allowing blockade of chronic extrasynaptic NMDAR activation. This discrimination was recently demonstrated definitively in a study on autaptic hippocampal cultures[148]. This study showed that the relative selectivity for extrasynaptic NMDARs is sensitive to the concentration of memantine used, as increasing the levels of memantine can lead to blockade of synaptic NMDARs (Figure 6b). Note, however, that it has been shown that memantine also preferentially antagonizes GluN2C- and GluN2D-containing NMDARs over GluN2A- and GluN2B-containing NMDARs under physiological levels of Mg^{2+} [149]. This should be taken into account when interpreting the effects of memantine in systems in which GluN2C/D levels are substantial.

Nevertheless, the usefulness of memantine in preferentially uncoupling the NMDAR from pro-death extrasynaptic NMDAR signalling has also been shown[39, 43]. For example, low doses of memantine not only block NMDAR-dependent neuronal death that is induced by toxic doses of NMDA without interfering with trans-synaptic NMDAR-dependent activation of neuroprotective pathways; it does so in a manner which does not induce neuronal degeneration *in vivo*, unlike 'conventional' antagonists such as MK-801[43]. The *in vivo* use of memantine to antagonize neurodegenerative pathways that were characterized *in vitro* has been elegantly demonstrated in the context of HD pathology[44, 132]. Treatment of YAC128

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mice with low dose memantine (1 mg/kg) from 2 months of age restored striatal phospho-CREB levels to a level similar to that in wild-type animals, and prevented the motor learning deficits that are normally apparent at 4 months of age[132]. A similar dosing regime also prevented striatal loss in YAC128 mice at 12 months and prevented motor deficits[44]. The protective effects of low-dose memantine were in sharp contrast with the effects of a much higher dose (30 mg/kg), which blocks all NMDARs: an exacerbation of striatal cell loss and motor deficits[44]. Given that memantine is a well-tolerated drug that has already been approved for clinical use, it will be of great interest to see whether clinical trials of memantine for HD reveal a reduction or delay of symptoms.

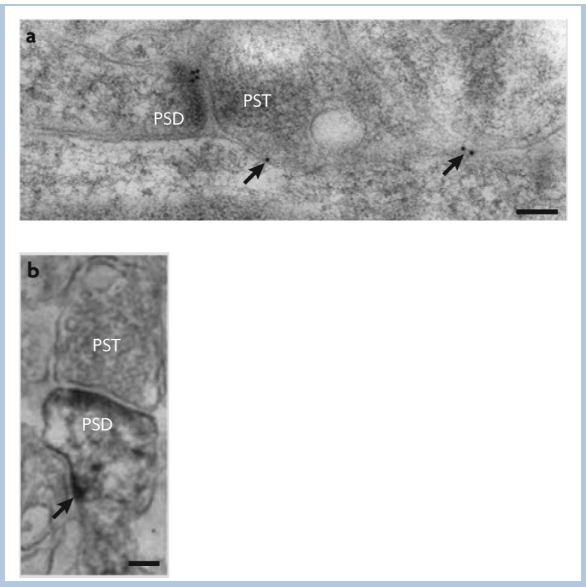
Concluding remarks

The survival and well-being of neurons requires synaptic activity and a dialogue between the synapse and the nucleus. Ca^{2+} signals, initiated by synaptic NMDARs, propagate inward to the nucleus and control genomic programmes that lead to a decrease in mitochondrial vulnerability, reduced expression of pro-apoptotic factors including caspases, and a strengthening of antioxidant defence systems. This form of acquired neuroprotection increases the 'neuronal survival reserve' that enables neurons to cope with harmful conditions in order to fulfil their function as computational units within neural circuits. Extrasynaptic NMDARs antagonize the pro-survival signalling of synaptic NMDARs by disturbing the communication between the synapse and the nucleus at various levels. Cell death associated with neurodegenerative diseases (including AD and HD) may be partly due to an imbalance of synaptic and extrasynaptic NMDAR signalling caused by synapse loss, failure to transduce Ca^{2+} signals from the synapse to the nucleus, or by redistributions of NMDARs from synaptic to extrasynaptic site. The antagonistic signalling of synaptic and extrasynaptic NMDARs provides a novel conceptual basis for future developments of neuroprotective therapies.

BOX1

Studying and defining synaptic and extrasynaptic NMDARs

Synaptic N-methyl-D-aspartate receptors (NMDARs) are classically defined as functional receptors that are activated by glutamate released during low frequency synaptic events [116, 150-153]. This could include low-frequency evoked events, spontaneous synaptic events and action potential-independent synaptic release of single quanta of glutamate. Extrasynaptic NMDARs — that is, receptors that are not activated during low-frequency synaptic events — can be found at various locations [6], including on the cell body, the dendritic shaft, the neck of the dendritic spine and also adjacent to the post-synaptic density (often referred to as perisynaptic) (see the figure, which shows examples of immuno-electron-micrograph (EM) images of extrasynaptic NMDARs on the dendritic shaft (A) and dendritic spine (B) in the hippocampal CA1 area of an adult rat ; the NMDARs are labelled with immunogold (A) or immunoperoxidase (B) using an NR1 antibody). Notably, in cultured neurons a proportion of NMDARs have the capacity to move into and out of synaptic sites [113, 151, 154], particularly GluN2B-containing NMDARs in young neurons [113] (although other studies have concluded that the mobility of GluN2B- and GluN2A-containing NMDARs is similar [151]). However, such NMDAR mobility was not observed in acute slices, raising the question as to whether mobility is more restricted in vivo [116].



To study signalling associated with synaptic and extrasynaptic populations of NMDARs, the receptors must be isolated for electrophysiological characterization, and stimulation paradigms should be able to preferentially activate either pool. Preferential activation of synaptic NMDARs in cultured hippocampal or cortical neurons can be initiated via pharmacological removal of the $GABA_A$ receptor-mediated inhibition from the network. This induces bursts of firing activity and results in tetrodoxin (TTX)-sensitive NMDAR-dependent Ca^{2+} transients [50]. A large portion of NMDARs are not activated by this stimulation paradigm and are presumed to be predominantly extrasynaptic NMDARs [38]. Other techniques, such as the stimulation of afferents, are more suited to slice preparations.

The general principle applied in studies of extrasynaptic NMDARs and the resultant downstream signalling is to irreversibly block synaptic receptors before bath-applying the agonist (glutamate or NMDA), so that the agonist will activate only the extrasynaptic receptors. Synaptic NMDARs can be blocked by selectively inducing synaptic glutamate release under conditions that activate synaptic NMDARs, but in the presence of the open channel blocker MK-801 (blockade is essentially irreversible over the timescale of most experiments). Brief pulses of high K^+ or episodes of firing activity, or simply allowing spontaneous action potential-dependent synaptic events can, in the presence of MK-801, induce open channel blockade of synaptic NMDARs [38, 43, 115, 155]. A slightly more stringent approach involves only blocking those NMDARs that are exposed to glutamate upon release of single quanta of glutamate [43]. The isolation of extrasynaptic NMDAR currents in hippocampal slice CA1 neurons can involve a similar strategy: MK-801 open-channel blockade of synaptic NMDARs activated by low-frequency stimulation of the stratum radiatum, followed by local uncaging of glutamate to activate extrasynaptic NMDARs [116]. Note that strategies aimed at selectively blocking synaptic NMDARs require confirmation that little or no activation of extrasynaptic NMDARs takes place over the timeframe of synaptic stimulation – such activation could be caused by astrocytic glutamate release or elevated ambient glutamate due to nearby cellular damage.

Acknowledgments

We thank Dr Karen Bell and Dr. C. Peter Bengston for comments on the manuscript. Work in the authors' laboratories is supported by the Wellcome Trust, the MRC, the BBSRC, the Royal Society (GEH) and by the Alexander von Humboldt Foundation, the ERC Advanced Grant, the Deutsche Forschungsgemeinschaft, the EU Network of Excellence NeuroNE, and the EU Project GRIPANNT (HB). GEH is a MRC Senior Non-Clinical Research Fellow. HB is a member of the Excellence Cluster *CellNetworks* at Heidelberg University.

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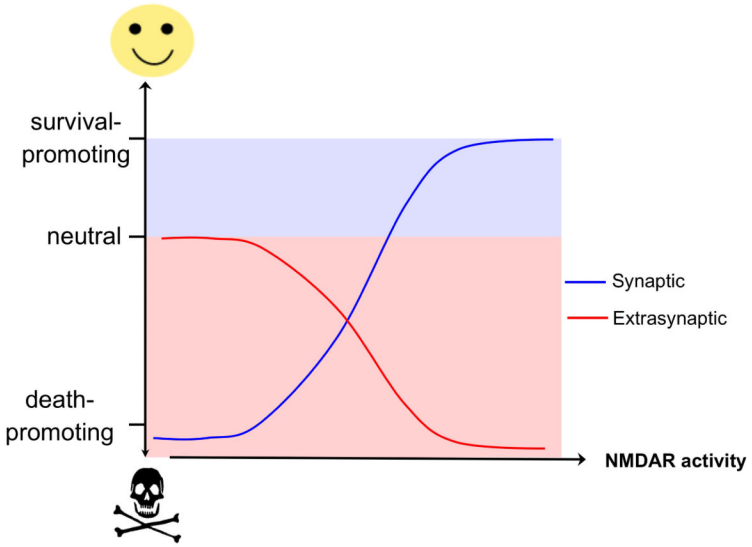

Figure 1. χ-shaped model of NMDAR-dependent excitotoxicity

The schematic illustrates the opposing effects of increasing synaptic and extrasynaptic N-methyl-D-aspartate receptor (NMDAR) activity on neuronal survival and resistance to trauma. Hypoactivity of synaptic NMDARs is harmful to neurons. Enhancing synaptic NMDAR activity triggers multiple neuroprotective pathways and this promotes neuronal survival. Low levels of activation of extrasynaptic NMDARs have no effects on neuronal survival but increasing the level of extrasynaptic NMDAR activity activates cell death pathways and exacerbates certain neurodegenerative processes, thus reducing neuronal survival.

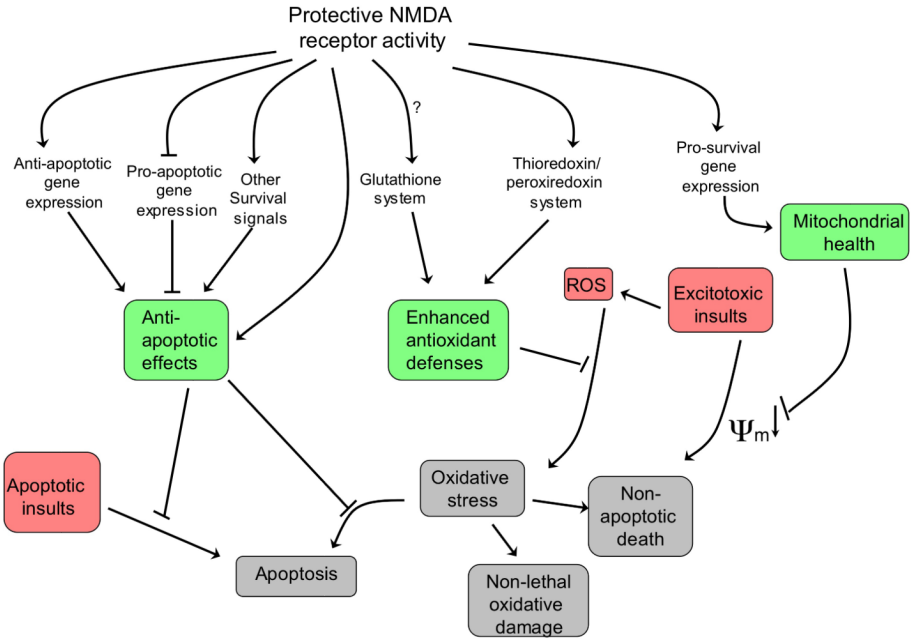

Figure 2. Neuroprotective pathways activated by synaptic NMDAR activity

Changes in gene expression underlie the long-lasting neuroprotection exerted by activation of synaptic N-methyl-D-aspartate receptors (NMDARs). Changes include both the up-regulation of protective genes — including anti-apoptotic genes, pro-survival genes and genes involved in antioxidant responses — and the transcriptional suppression of pro-death genes. This restricts the apoptotic potential of the neuron, reduces vulnerability of mitochondria to insult-induced depolarization, and boosts intrinsic antioxidant defences (these three effects are shaded in green). These changes boost neuronal resistance to a range of traumata, including apoptotic, excitotoxic and oxidative insults (shaded in red), and thereby prevent harmful outcomes (shaded in grey) such as apoptotic and non-apoptotic cell death and cell dysfunction.ψm refers to mitochondrial membrane potential. ROS, reactive oxygen species.

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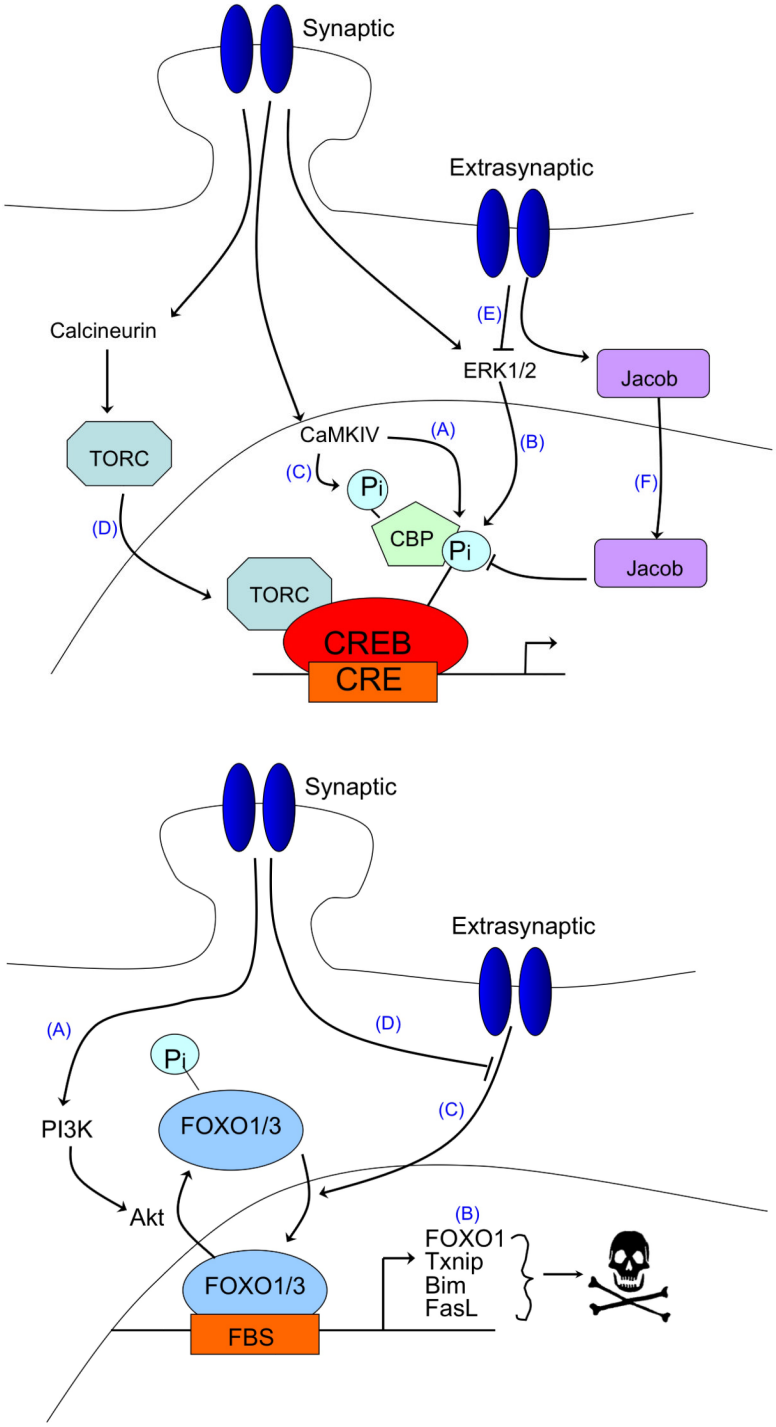

Figure 3. Opposing effects of synaptic vs. extrasynaptic NMDAR signalling on gene expression

A. Opposing effects of synaptic vs. extrasynaptic NMDAR signalling on CREB-dependent gene expression. The activation of cyclic-AMP response element binding protein (CREB)-dependent gene expression by synaptic N-methyl-D-aspartate receptor (NMDAR) activity is a multi-step process. CREB must be phosphorylated at serine-133 in order to recruit its coactivator CREB binding protein (CBP) [52], [54]; this phosphorylation is

mediated by the fast-acting nuclear CaM kinase pathway (A) and the slower acting, longer lasting Ras-ERK1/2 pathway (B) [50], [156], [157], both of which are promoted by activation of synaptic NMDARs CBP is subject to Ca^{2+}-mediated transactivation by nuclear Ca^{2+} dependent CaM kinase IV [58], [86], which phosphorylates CBP at serine-301 (C) [158]. In addition, nuclear translocation of transducer of regulated CREB activity (TORC) is a key step in CREB activation. Synaptic NMDAR-induced Ca^{2+} signals promote TORC import into the nucleus via calcineurin-dependent dephosphorylation (D) [159], [160]. TORC acts at least in part by assisting in the recruitment of CBP to CREB [161] (not shown). In contrast to these CREB-activating signals of synaptic NMDARs, extrasynaptic NMDARs suppress CREB activity [38], [89] through inactivation of the Ras-ERK1/2 pathway (E) [41] and the nuclear translocation of Jacob, which promotes CREB dephosphorylation (F) [90].

B. Opposing effects of synaptic vs. extrasynaptic NMDAR signalling on FOXO-dependent gene expression. Synaptic NMDAR activity suppresses FOXO activity by promoting the Akt-mediated phosphorylation and nuclear export of FOXOs (A) [43], of which FOXO1 and FOXO3 are the predominant neuronal subtypes. FOXO1 is also regulated transcriptionally by FOXOs [78] and thus signals that cause FOXO export also result in FOXO1 transcription being suppressed. By contrast, bath activation of NMDARs, which also triggers extrasynaptic NMDAR activity, stimulates FOXO nuclear import (B), an event that contributes, through promoting transcription of pro-death genes, to excitotoxic cell death. [77] Synaptic NMDAR activity can exert a long-lasting block on this import signal (C) [77], the mechanistic basis of which remains unclear.

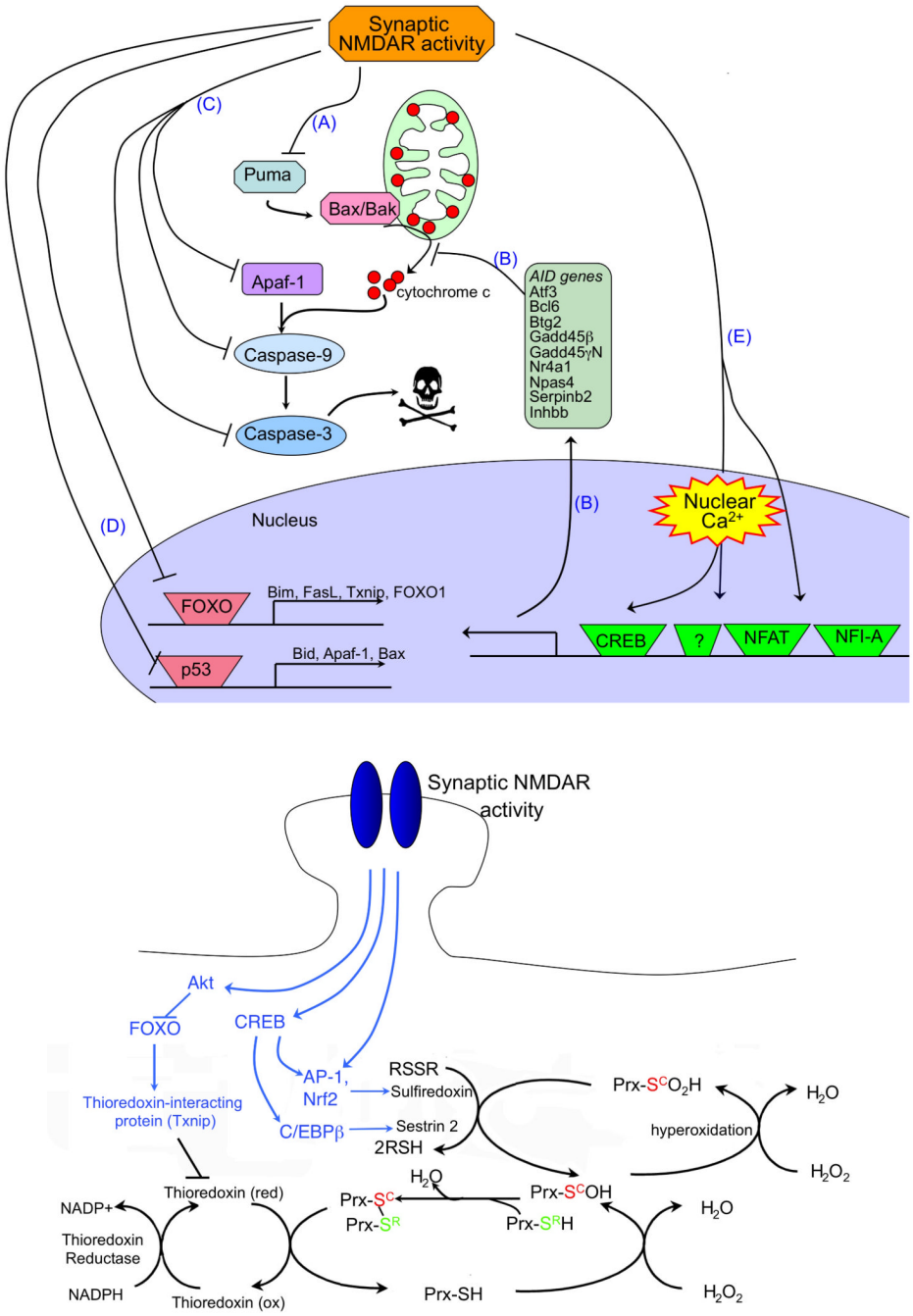

Figure 4. Synaptic NMDAR-dependent transcriptional changes have both anti-apoptotic and anti-oxidant effects

A) Synaptic NMDAR signalling suppresses the intrinsic apoptosis cascade at multiple levels. Synaptic N-methyl-D-aspartate receptor (NMDAR) activity protects neurons against apoptosis in a manner that is independent of the type of cellular insult because it suppresses the core apoptotic pathway. Transcriptional suppression of the BH3-only domain gene *Puma* by activation of synaptic NMDARs is a key event upstream of cytochrome c release (A) [66], [67]. Up-regulation of a battery of nuclear Ca^{2+}-regulated genes (green box) is also strongly protective [47], [51] and probably also acts upstream of cytochrome c release, as it inhibits

insult-induced mitochondrial membrane depolarization (B). Downstream of cytochrome c release, transcriptional suppression of components of the caspase cascade, such as Apaf-1, Caspase-9 and Caspase-3, also slows the apoptotic process (C) or raises the amount of mitochondrial cytochrome c release that is required to initiate apoptosis (as cytochrome c's cytoplasmic target is Apaf-1) [67]. Synaptic NMDAR activity also induces inactivation of the pro-death transcription factors FOXO and p53 (D) [43], [66], [78], and the subsequent suppression of the genes whose expression they regulate. Finally, transcription factors that have been shown to be important targets for NMDAR-dependent pro-survival signals are shown, namely CREB, NFAT and NFI-A (E) [47], [72], [73].

B) Synaptic NMDAR signalling boosts intrinsic antioxidant defences. A schematic showing the effect of synaptic activity-induced changes in transcription on the thioredoxin (Trx)-peroxiredoxin (Prx) system. The dotted arrows indicate activity-dependent signalling events, which are collectively shaded in green. Briefly, reduction of peroxide levels is executed via the transfer of electrons from NADPH to peroxides through the redox-active sulfhydryl (-SH) groups of thioredoxin reductase, Trx and Prxs (the major two-cysteine Prx sub-type is shown). The catalytic redox-active cysteine residue of Prx (S^C, red) reduces peroxide, and is oxidized to cysteine sulfenic acid (S^COH). The resolving cysteine (S^R, green) of a different Prx molecule then forms a disulfide bond with Prx-S^COH, thereby eliminating H_2O. This intermolecular disulfide bond is then reduced by Trx, whose two-cysteine active site is reduced by Trx reductase. However, if levels of peroxide are too high, the – S^COH group becomes hyperoxidized to $–SO_2H$ (cysteine sulfinic acid). $–SO_2H$ is not a substrate for the resolving cysteine $–S^CH$ or for Trx. Instead, sulfiredoxin (and possibly sestrin 2) catalyses the reduction of hyperoxidized Prx-SO_2H, returning it to the Trx cycle. Synaptic activity triggers the events shown to both enhance Trx activity and boost the reduction of hyperoxidized Prxs. These include the transcriptional activation of sulfiredoxin and sestrin 2, and the transcriptional suppression of *Txnip*, the gene encoding a the Trx inhibitor thioredoxin-interacting protein (Txnip). Bim (bcl2-interacting mediator of cell death); Fasl (fas antigen ligand); Apaf1 (apoptotic peptidase activating factor 1); CREB (cAMP responsive element binding protein); NFAT (nuclear factor of activated T-cells); NFI-A (nuclear factor I/A); Atf3 (activating transcription factor 3); Bcl6 (B-cell leukemia/lymphoma 6); Btg2 (B-cell translocation gene 2); Gadd45beta, GADD45gamma (growth arrest and DNA damage induced gene 45 gamma and beta, respectively); Nr4a1 (nuclear receptor subfamily 4, group A, member 1); Npas4 (neuronal PAS domain protein 4); Inhbb (Inhibin beta-A).

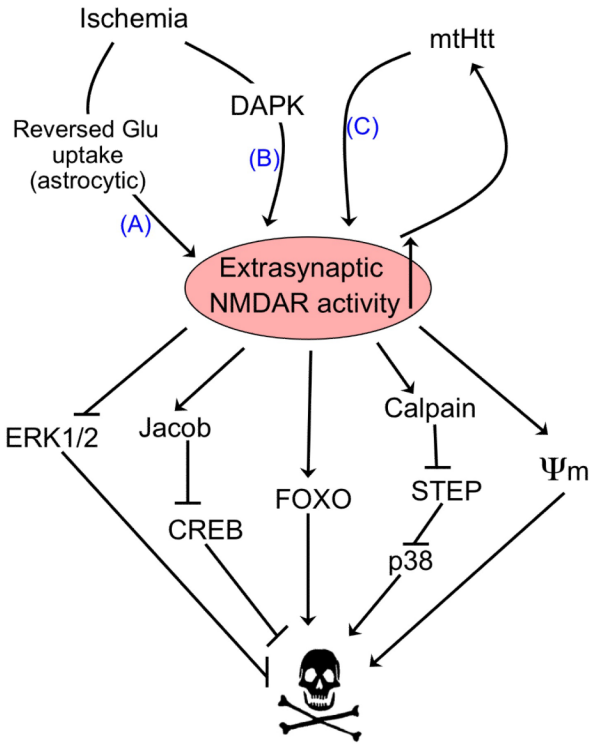

Figure 5. Activators and effectors of extrasynaptic NMDAR activity

Ischaemia results in activation of extrasynaptic N-methyl-D-aspartate receptor (NMDAR) activation through reversed glutamate uptake from astrocytes (A) [130]. Extrasynaptic currents are also preferentially enhanced by ischaemia-induced death-associated protein kinase (DAPK) activation (B) [131], as well as by mutant Huntingtin (mtHtt) in a mouse model of Huntington's disease (C) [132]. Increased extrasynaptic (but not synaptic) NMDAR activity in turn preferentially activates a number of pro-death pathways. ψm refers to mitochondrial membrane potential, which is disrupted by extrasynaptic NMDAR activity.

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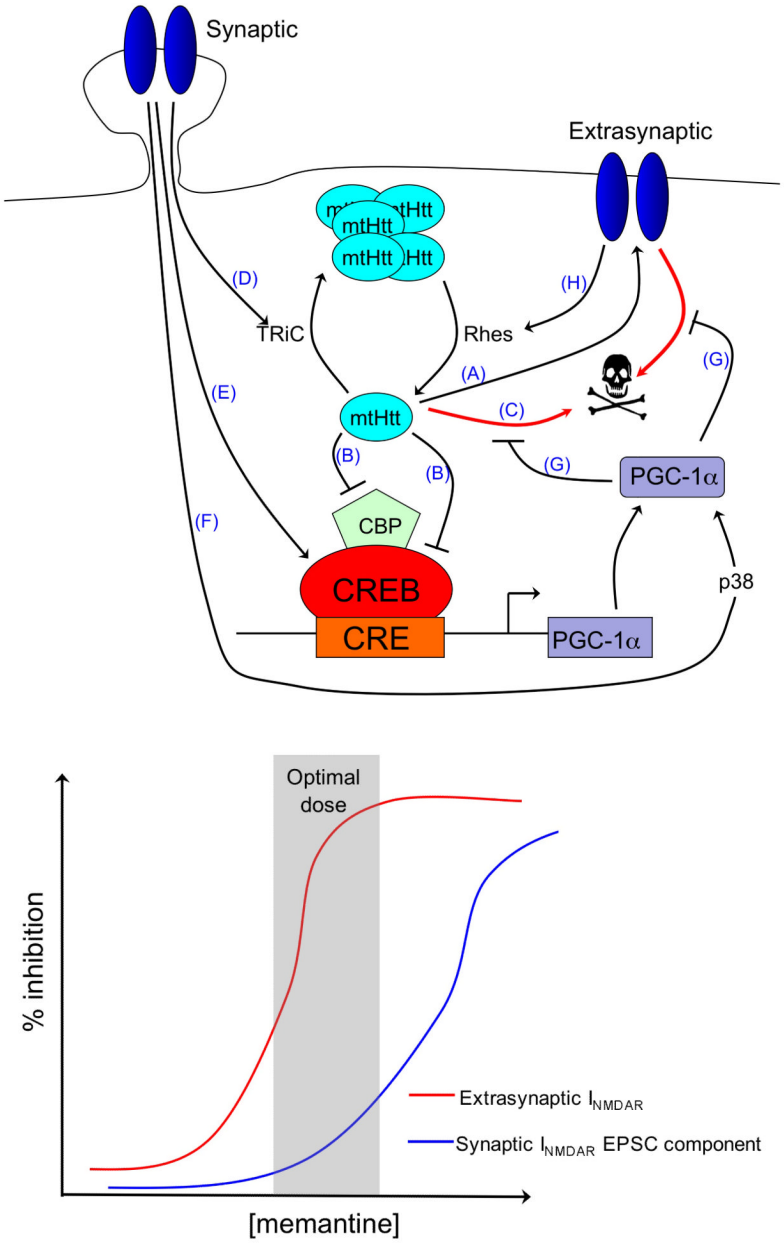

Figure 6. Clinical relevance of the balance between synaptic and extrasynaptic NMDAR activity

A. The balance of synaptic vs. extrasynaptic NMDAR signalling influences HD pathology. Mutant Huntingtin (mtHtt) promotes neuronal dysfunction and death by enhancing the expression and activity of extrasynaptic N-methyl-D-aspartate receptors (NMDARs) (A) [132] and by directly blocking CREB/CBP-dependent PGC-1α transcription (B) [134, 139, 140], as well as through other toxic mechanisms (C). Synaptic NMDAR activity opposes mtHtt toxicity by promoting TRiC-dependent aggregation of mtHtt into non-toxic inclusions (D) [44]. Synaptic NMDAR activity also activates CREB-dependent PGC-1α transcription (E) [44, 101], and enhances PGC-1α activity post-translationally via p38 (F)[101]. PGC-1α itself protects neurons against mtHtt toxicity and extrasynaptic-NMDAR-dependent excitotoxicity (G) [44, 101, 162]. Extrasynaptic NMDAR activity reduces the formation of non-toxic mtHtt inclusions by enhancing the expression of Rhes, a GTPase

known to sumoylate and disaggregate mtHtt (H) [44]. In addition, extrasynaptic NMDAR signals suppress CREB-dependent gene expression [38] (not shown).

B. Effect of memantine on NMDARs. Low doses of memantine preferentially block the chronic activation of extrasynaptic NMDARs (red line) by elevated levels of glutamate, which may occur as a result of ischaemia or impaired glutamate homeostasis associated with other disease processes. Low levels of memantine spare the trans-synaptic activation of synaptic NMDARs (blue line). This specificity of the effect of memantine has been demonstrated electrophysiologically [126, 147] as well as by studying downstream pro-death and pro-survival signalling [39, 43, 44, 132].